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A Systematic Review of the Outbreak of *Elizabethkingia Anophelis*

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A Systematic Review of the Outbreak of *Elizabethkingia Anophelis*

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Abstract

The recent outbreak of *Elizabethkingia anophelis* in the Midwestern states of USA caused a number of deaths. Notably, these deaths occurred due to *E. anophelis* causing neonatal meningitis, bacteraemia, sepsis, blood stream infections and respiratory infections. These infections may pose serious threats to public health. This systematic review is meant to develop a deeper insight into the current status of *E. anophelis* related evidence and to highlight areas that need further research. Reviewing the existing literature will help other researchers to identify and address the knowledge gaps. Various free access databases such as Google Scholar, Scopus, PubMed, and Science Direct were employed for literature survey. All articles published since 2011, when the outbreak was reported for the first time, were consulted for this systematic review. Research related to this subject is in its earlier stages and little information is currently available. Future studies must focus on the molecular basis, control, prevention, and therapeutics of *E. anophelis* to mitigate its increasing risk. This review is meant to provide baseline data for future research. Scientific community must carry out research on the infections caused by *E. anophelis* mosquito, else it may result in a disastrous outbreak.

Introduction

Genus *Elizabethkingia* is a part of family *Flavobacteriaceae* and phylum *Bacteroidetes*. It is a non-motile, ubiquitous, and aerobic bacterium mostly found in the gut of *Anopheles* mosquito and colonizes the human respiratory tract. Four different species that belong to the genus *Elizabethkingia* are *E. miricola*, *E. meningoseptica*, *E. endophytica*, and *E. anophelis*.

E. meningoseptica is a nosocomial pathogen that affects patients on hemodialysis [1, 2] and is responsible for bacteremia [3], septicemia [4],

endophthalmitis [5], and meningitis [2, 6-9]. *E. miricola* has the potential to cause ventilator associated pneumonia, sepsis, and bacteremia [10, 11]. *E. endophytica* was isolated from *Zea mays* [12]. Phylogenetic analysis revealed that *E. anophelis* is different from the closely related species *E. miricola* and the related group *E. meningoseptica* (Figure 1).

E. anophelis is a gram-negative bacteria isolated from the midgut of the anopheline mosquito.

Studies involving a three-year long outbreak in Taiwan spanning the time period 2015-2018 resulted in the

identification of a specific *E. anophelis* strain. Transmission mechanism patterns in 26 patients were studied using Pulsed-field gel electrophoresis (PFGE) and through complete genome sequencing [14].

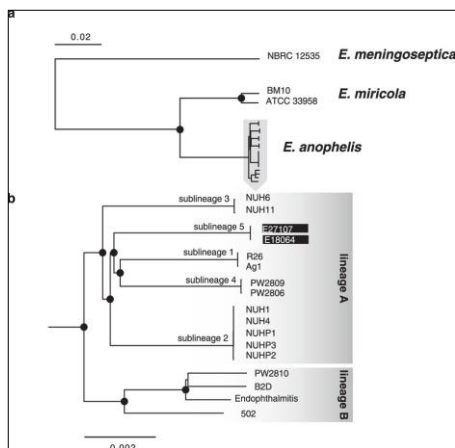


Figure 1. Phylogenetic tree of Elizabethkingia species [13]

A study was carried out in Saudi Arabia of 27 patients hospitalized between June 2013 and May 2019 and suspected of having *Chryseobacterium* / Elizabethkingia spp infection. Blood culture studies showed that *Elizabethkingia* spp indeed was the most prevalent among the pathogens isolated [15]

In another study undertaken in Singapore from 2009 to 2017, 79 blood culture

isolates were probed. PCR assisted results showed that 78/79 of these isolates were of *E. anophelis*, manifesting the overwhelming dominance of the strain under review [16].

E. anophelis infection was responsible for a public health crisis in the US states of Michigan, Illinois and Wisconsin with 65 confirmed cases and 20 deaths as of June 2016 [17]. The high mortality rate associated with this infection has raised serious concern during the past few years (Figure 2).

Strains of Elizabethkingia are usually found in fresh and marine environments. Mostly, immunocompromised individuals acquire the *E. anophelis* infection during hospital stay [18, 19]. Most people acquire bloodstream infections but respiratory infections were also reported in some cases during 2015-16 *E. anophelis* outbreak. *Elizabethkingia* is resistant to many antibiotics [16]. According to some clinicians, *E. anophelis* bacteria are susceptible to antibiotics such as fluoroquinolones and rifampin; therefore, treating patients with a combination of antibiotics may improve the outcome [20]. Common symptoms include the shortness of breath, cough, chills, fever, cellulitis, headache, and joint pain. Evidence of vertical transmission was reported recently but the proper transmission path and different modes of transmission are still vague [21-24].

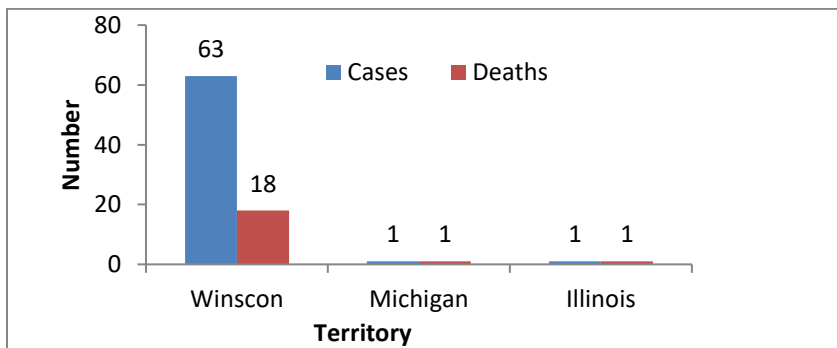


Figure 2. Confirmed cases and deaths during the recent deadly outbreak of *E. anophelis* in the Midwestern states

This systematic review is meant to develop a deeper insight into the current status of *E. anophelis* related research and to highlight areas that need further research. Reviewing the existing literature will help other researchers to identify and address the knowledge gaps.

2. Methodology

2.1. Literature Survey and Data Screening

Various free access databases such as Google Scholar, Scopus, PubMed, and Science Direct were employed for the literature survey. Little research has been conducted on *E. anophelis*. We used different keywords such as *E. anophelis* infection, *E. anophelis* transmission, *E. anophelis* symptoms, *E. anophelis* strain diversity, *E. anophelis* future prospects, and *E. anophelis* treatment. Our comprehensive search yielded 17 records.

2.2. Quality Assessment

Eligible publications included all research articles or original studies related to *E. anophelis* from 2011 (when it was initially reported) to 2016. The authors analyzed the available literature independently and removed the duplicates.

2.3. Data Synthesis

A total of 13 articles were included and Microsoft Excel spreadsheet was employed to record the relevant information such as the author(s), method, key findings, and conclusion.

3. Results

A record of primary literature published since *E. anophelis* was first reported was compiled after a thorough literature search.It included peer-reviewed articles available in the English language. Table 1 depicts the key findings, method, study areas, and study design of all the documents that met the inclusion criteria.

Table 1. Currently Available Studies Related to *E. anophelis*

Ref.	Methodology	Key Findings	Conclusion
1	Pathogenesis associated features and phylogenetic relationships of two African neonatal meningitis <i>E. anophelis</i> isolates compared with <i>Elizabethkingia</i> isolated from other sources and regions [13].	Distinct sublineages observed in African <i>E. anophelis</i> were genetically related. Specific resistance genes acquired as a result of horizontal transfer were also observed in African isolates.	The emerging pathogen <i>Elizabethkingia</i> is dynamically evolving over time.
2	Complete circularized genome sequences of 4 strains collected during <i>E. anophelis</i> outbreak were studied [25].	Mapping of outbreak strains showed similarity with the genome of the strain CSID_3015183678. Ordered arrangement was observed at three segments A, B, and C belonging to the position 3929927.	Complete gene sequences were deposited at GenBank under BioProject no. PRJNA315668.
3	The molecular and clinical epidemiology of <i>Elizabethkingia</i> -like species isolated from	16S rRNA based gene sequencing revealed that out of the total 45 episodes of bacteremia associated	<i>E. anophelis</i> is the predominant cause of <i>Elizabethkingia</i> bacteremia and the

Ref.	Methodology	Key Findings	Conclusion
	bacteraemia patients admitted in 5 regional hospitals of Hong Kong was analyzed [26].	with Elizabethkingia-like species, <i>E. anophelis</i> , <i>E. meningoseptica</i> , <i>E. miricola</i> , and other diverse genera / species were responsible for 17, 1, 3, and 24 episodes, respectively.	morbidity and mortality associated with this life-threatening condition.
4	All four species of <i>Elizabethkingia</i> were sequenced [27].	Results revealed that <i>E. endophytica</i> and <i>E. anophelis</i> belong to genomospecies 1, whereas <i>E. miricola</i> is similar to genomospecies 2.	Complete genome sequences were deposited at GenBank under BioProject no. PRJNA301708.
5	Researchers sequenced <i>E. anophelis</i> from Asian malaria vector <i>Anopheles stephensi</i> [28].	This whole genome shotgun project was deposited at GenBank under the accession no. LFKT00000000.	This genome sequence provided the baseline data needed to analyze host-microbe interactions in mosquitoes.
6	Researchers examined the number of <i>E. anophelis</i> in midgut and its physiological requirements using selectable markers, reporter systems (green fluorescent protein [GFP] and NanoLuc), and transposons that function in <i>E. anophelis</i> for genetic manipulation. They also examined <i>PompA</i> based flavobacterial expression system integrated into <i>E. anophelis</i> to enhance promoter activity and it led to the increased production of NanoLuc and GFP [29].	A 71%, 82% and 3% infection rate was determined with <i>A. gambiae</i> , <i>A. stephensi</i> , and <i>A. triseriatus</i> , respectively when fed with NanoLuc-tagged cells at the larval stage. Arginine was found to be an important amino acid for <i>E. anophelis</i> whose growth was promoted by animal erythrocytes <i>in vivo</i> and <i>in vitro</i> , suggesting that erythrocyte lysis in the mosquito midgut provides nutrients.	The study revealed the molecular manipulation and interaction of <i>E. anophelis</i> with mosquito hosts and showed that <i>E. anophelis</i> adapts to different mosquito midgut environments variously.
7	Sequencing of <i>E. anophelis</i> NUHP1 was done and its response to	Results indicated the presence of 4,369,828 base pairs with long circular genome	Heme uptake and production of siderophore act as key players of stress response and

Ref.	Methodology	Key Findings	Conclusion
	oxidative stress was also assessed [30].	containing 4,141 predicted coding sequences. Sequence analysis also revealed that <i>E. anophelis</i> possesses an organized system stress response and iron scavenging. We further showed that hemoglobin facilitates growth, hydrogen peroxide tolerance, cell attachment and biofilm formation of <i>E. anophelis</i> NUHP1.	virulence of <i>E. anophelis</i> .
8	JM-87(T) bacterial strain was isolated from corn plant and studied for taxonomic classification [12].	The bacteria appeared rod-shaped and gram-negative. Based on 16S rRNA gene sequences, isolate exhibited 99.1%, 97.8% and 97.4% similarity to <i>E. anophelis</i> , <i>E. meningoseptica</i> and <i>E. miricola</i> , respectively.	JM-87(T) proved to be a novel species labelled as <i>E. endophytica</i> .
9	R26 ^T and Ag1, two different strains of <i>E. anophelis</i> isolated from different strains of <i>A. gambiae</i> were sequenced [31].	Both strains of bacteria were identical. Different TonB dependent transporters with different substrate specificities were observed in <i>E. anophelis</i> genome. <i>E. anophelis</i> genome also contains several genes with broad antibiotic resistance, genes that encode efflux pumps and β -lactamases and genes important for mosquito's carbohydrate metabolism. <i>E. anophelis</i> encodes various hemolysins that increase the hemolytic activity leading to erythrocytes' digestion in the mosquito gut. Antioxidant genes and	The study elucidated functional characteristics and the symbiotic relationship of bacterium with the mosquito host.

Ref.	Methodology	Key Findings	Conclusion
		OxyR regulon provide defense against the oxidative stress associated with blood digestion.	
10	Researchers used rapid genome sequencing to examine 3 isolates of <i>E. anophelis</i> obtained from 1 mother and 2 neonates who had chorioamnionitis and meningitis, respectively [24].	Genomics revealed that bacteria were transmitted from the mother to her neonates. Genome of 2 strains, that is, HKU37 and HKU38 were identical to each other but different from the third strain HKU36, thus excluding a clonal outbreak.	The study suggested the vertical transmission of <i>E. anophelis</i> associated infections.
11	Whole genome sequencing of seven isolates of <i>E. anophelis</i> were collected from different hospitals and compared with five <i>Elizabethkingia</i> spp. Genomes were available over NCBI. Researchers applied the pan-genomic approach for the identification of core- and pan-genome for the <i>Elizabethkingia</i> genus [32].	The genome of <i>E. anophelis</i> strains were identical to <i>E. anophelis</i> Ag1 and R26 strains isolated from the malaria mosquito vector <i>Anopheles gambiae</i> .	The results of the study highlighted the nosocomial transmission of <i>E. anophelis</i> infection.
12	Researchers isolated R26(T) from the midgut of the mosquito <i>Anopheles gambiae</i> and studied its growth properties, antibiotic resistance characteristics, and taxonomic allocation [23].	Isolates appeared as rod-shaped gram-negative cells. Optimum growth of bacteria was observed at 30-31°C and 37°C. Bacteria showed resistance against streptomycin, chloramphenicol, kanamycin, tetracycline, and ampicillin. R26(T) was 98.2% similar to <i>E. miricola</i> GTC 862(T) and 98.6% similar to <i>E. meningoseptica</i> ATCC 13253(T) based on 16S	The study proposed that strain R26(T) represented a novel species that was named <i>E. anophelis</i> .

Ref.	Methodology	Key Findings	Conclusion
		rRNA gene sequence analysis.	
13	Authors presented the case study of an 8-year old girl brought to Complexe Pédiatrique in Bangui, Central African Republic in March 2011 [33].	Strain was identified as <i>E. meningoseptica</i> using API 20NE system strip. Phylogenetic analysis based on 16S rRNA gene exhibited that the isolate belonged to <i>E. anophelis</i> .	<i>E. anophelis</i> associated meningitis was reported for the first time in Arfica in 2011.
14	We presented the draft genome sequences of two strains of <i>E. anophelis</i> , R26 ^T and Ag1, which were isolated from the midgut of the malaria mosquito <i>Anopheles gambiae</i> [34].	CLC Genomics Workbench v.4.9 based de novo assembly generated 51 contigs, totaling 4.05 Mbp and DNASTAR NGen v 10.0 based de novo assembly of R26 ^T genomic reads (652 Mbp) yielded 66 contigs, totaling 4.03 Mbp with an average GC content of 35.4%. NCBI Prokaryotic Genome Automatic Annotation Pipeline revealed 3,648 protein coding sequences CDS and 38 RNA genes in Ag1 and 3,687 protein coding sequences (CDS).	The draft genome sequences of strains Ag1 and R26 ^T are available in DDBJ/EMBL/Gen Bank under the GenBank accession numbers AHHG000000000 and ANIW000000000, respectively.

4. Discussion

In this review, 14 records were identified that included 11 original research articles and 2 case reports published between 2011 and 2016. These studies highlighted the vector potential of mosquitoes for the transmission of *E. anophelis* to humans.

There is strong evidence that *E. anophelis* transmits from mother to fetus and this infection is currently circulating in the US states of Michigan, Illinois and Wisconsin. The prevalence of *E. anophelis* is much higher than *E. meningoseptica* and *E. miricola* [25]. Previous studies showed that *E. anophelis* associated bacteremia carries high morbidity and mortality [26]. Accumulating evidence suggests that *E.*

anophelis is misidentified as *E. meningoseptica* but MALDI-TOF MS is the most appropriate choice for the accurate and rapid diagnosis of *E. anophelis* infection. The complete genomic sequences of four different strains collected during a recent outbreak of 2015-16 were deposited to GenBank under the BioProject no. PRJNA315668 [26, 28]. Previously, complete genomic sequences of two strains R26^T and Ag1 isolated from the midgut of the malaria mosquito *Anopheles gambiae* were available under the GenBank accession numbers ANIW000000000 and AHHG000000000, respectively. Likewise, the genomic sequence of *E. anophelis* strain EaAs1 isolated from the Asian

malaria mosquito *Anopheles stephensi* was deposited at GenBank under the accession no. LFKT00000000 [27].

A study demonstrated the molecular basis of *Elizabethkingia* infections and host mosquito interactions and also introduced the techniques used for the integration of foreign DNA into the chromosome and the expression of the gene of interest in commensal *Elizabethkingia* [29, 35]. This study provided future avenues for the development of novel biocontrol agent diseases caused by mosquitoes. The reporter strain, specifically that was GFP-based or NanoLuc-based, allowed the understanding of bacterial infection, *in vivo* cell localization, and gene regulation [35].

Immunocompromised patients are known to be mostly infected by the genus *Elizabethkingia* and a number of new species of this genus were reported in the last decade. *E. anophelis* is the most prevalent species of this genus. This genus of pathogen is sensitive to minocycline; however, it is resistant to β -lactam inhibitors, aminoglycosides, β -lactams, and carbapenems.

5. Conclusion

To conclude, *E. anophelis* related research is in its initial stages. This review identified the knowledge gap with respect to therapeutics, pathogenesis, transmission, phylogenetics, and molecular biology of the infection.

Conflict of Interest

The authors declare no conflict of interest

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